A41, A42 with C28, U28, or G28, or U41 and U42 with respect to the position numbering of +85 sgRNA or guide architecture.

[0013] In some embodiments, the modification of architecture is replacing bases in stemloop 2 with respect to the position numbering of +85 sgRNA or guide architecture.

[0014] In some embodiments, the "acuu" and "aagu" bases in stemloop2 are replaced with complimentary GC-rich regions of 4 nucleotides

[0015] In some embodiments, complimentary GC-rich regions of 4 nucleotides are "cgcc" and "gcgg".

[0016] In some embodiments, complimentary GC-rich regions of 4 nucleotides are "cgcc" and "gcgg".

[0017] In some embodiments, at least one loop of the sgRNA or guide RNA is further modified by the insertion of distinct RNA sequence(s) that bind to one or more adaptor proteins, and wherein the adaptor protein is associated with one or more functional domains.

[0018] In some embodiments, the insertion of distinct RNA sequence(s) that bind to one or more adaptor proteins is an aptamer sequence or two or more aptamer sequences specific to the same or different adaptor protein(s).

[0019] In some embodiments, the adaptor protein comprises MS2, PP7, Qβ, F2, GA, fr, JP501, M12, R17, BZ13, JP34, JP500, KU1, M11, MX1, TW18, VK, SP, FI, ID2, NL95, TW19, AP205, ϕCb5, ϕCb8r, ϕCb12r, ϕCb23r, 7s, PRR1.

[0020] In some embodiments, the functional domain is a transcriptional activation domain, a transcriptional repressor domain or comprises a nuclease domain.

[0021] In an aspect, the invention provides a non-naturally occurring or engineered composition comprising the present guide according to any preceding claim and a CRISPR enzyme.

[0022] In some embodiments, the CRISPR enzyme is Cas9.

[0023] In some embodiments, the CRISPR enzyme is Sa Cas9.

[0024] In some embodiments, CRISPR enzyme is Sp Cas9.

[0025] In some embodiments, the CRISPR enzyme is associated with one or more functional domains.

[0026] In some embodiments, the functional domain associated with the CRISPR enzyme is a transcriptional activation domain, a transcriptional repressor domain or comprises a nuclease domain.

[0027] In some embodiments, the cell is a eukaryotic cell. In some embodiments, the eukaryotic cell is a mammalian cell. In some embodiments, the mammalian cell is a human cell or a mouse cell.

[0028] In an aspect, the invention provides a polynucleotide encoding the present guide (e.g. sgRNA).

[0029] In an aspect, the invention provides a vector comprising the polynucleotide operably linked to a suitable promoter. In some embodiments, the vector also comprises a further polynucleotide encoding a CRISPR enzyme, optionally a Cas9.

[0030] In an aspect, the invention provides a vector system, comprising one of the present vectors and a second vector comprising a polynucleotide encoding a CRISPR enzyme, optionally a Cas9.

[0031] In an aspect, the invention provides a transformant organism or model transformed with any one of the vectors to thereby express the guide RNA or optionally sgRNA and, optionally, the CRISPR enzyme.

[0032] In an aspect, the invention provides a method of modifying a genomic locus of interest to alter gene expression in a cell by introducing into the cell the composition, polynucleotide or vectors described herein.

[0033] In some embodiments, the guide RNA or optionally sgRNA comprises, in a tandem arrangement:

[0034] I. a guide sequence, which is capable of hybridizing to a sequence of the target nucleic acid to be manipulated;

[0035] II. a tracr mate sequence, comprising a region of sense sequence;

[0036] III. a linker sequence; and

[0037] IV. a tracr sequence, comprising a region of antisense sequence which is positioned adjacent the linker sequence and which is capable of hybridizing with the region of sense sequence thereby forming a stem-loop.

[0038] The invention, in its various aspects as described herein, is based on a number of surprising discoveries in relation to RNA components of CRISPR-Cas systems comprising a Staphyloccocus *aureus* Cas9 enzyme.

[0039] In an aspect the invention provides a composition as herein discussed which is a non-naturally occurring or engineered CRISPR-Cas system dual guide RNA molecule (dgRNA) capable of effecting the manipulation of a target nucleic acid within a prokaryotic or eukaryotic cell when in complex within the cell with a CRISPR enzyme comprising a *Staphyloccocus aureus* Cas9 enzyme (SaCas9); the dgRNA comprising:

[0040] I. a guide RNA molecule (e.g., a chimeric RNA or optionally sgRNA) comprising, in a tandem arrangement:

[0041] a) a guide sequence, which is capable of hybridizing to a sequence of a target nucleic acid to be manipulated; and

[0042] b) a tracr mate sequence, comprising a region of sense sequence; and

[0043] II. a tracr RNA molecule, comprising a region of antisense sequence which is capable of hybridizing with the region of sense sequence of the tracr mate sequence;

wherein the guide sequence comprises a length of 21 or more nucleotides.

[0044] When said chimeric RNA molecule and said tracr RNA molecule are present within the cell, the region of antisense sequence is hybridized to the region of sense sequence thereby forming the dgRNA molecule; and wherein when said dgRNA molecule binds within the cell to the CRISPR enzyme so forming a CRISPR-Cas complex, the guide sequence hybridizes to a sequence of the target nucleic acid thereby directing sequence-specific binding of the CRISPR/Cas complex to the target nucleic acid, whereupon said sequence of said target nucleic acid is manipulated by the CRISPR enzyme of the complex.

[0045] In an aspect the invention also provides a composition as herein discussed which is a non-naturally occurring or engineered CRISPR-Cas system chimeric single guide RNA molecule (sgRNA) capable of effecting the manipulation of a target nucleic acid within a prokaryotic or eukaryotic cell when in complex within the cell with a